# Chapter 18

# *Purification of Muscle Actin*

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## I. Introduction

The opportunity to study the molecular events responsible for muscle contraction and cell motility was made possible over 40 years ago by Banga and Szent-Györgyi (1941) and by Straub (1942), who discovered myosin and actin in the extracts of rabbit skeletal muscle. Actin was first isolated when Straub (1942)



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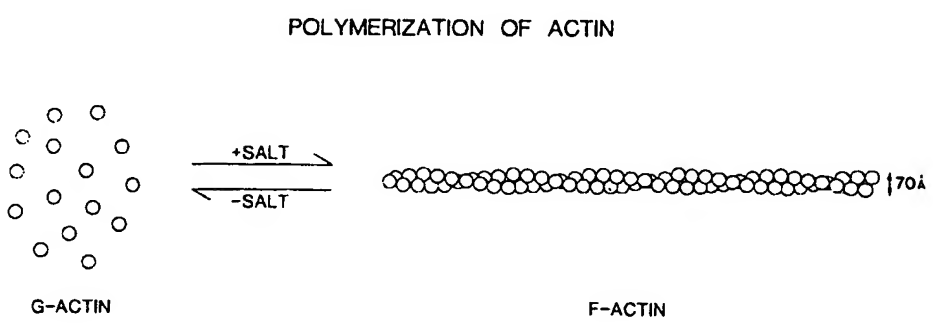


FIG. 1. Assembly of muscle actin. G-actin containing 1 ATP and 1 tightly bound divalent cation per monomer assembles in the presence of salt into 70-Å-diameter filaments of F-actin.

separated the viscous protein from an actomyosin preparation. Subsequent work (Straub, 1943) revealed that actin could be obtained in a nonviscous state (G-actin) by extracting muscle with a low ionic strength buffer, and that addition of salt induced conversion to a viscous form called F-actin (Fig. 1). An improved procedure by Straub and colleagues then incorporated a step that denatured muscle proteins not stabilized in an actomyosin complex; minced muscle was dehydrated with acetone before the actin extraction step (Straub, 1943; Feuer *et al.*, 1948). After the discovery that bound ATP was important for maintaining the functional integrity of actin (Laki *et al.*, 1950; Mommaerts, 1951; Straub and Feuer, 1950), the stability of isolated actin was enhanced by inclusion of ATP in the extraction buffers, but the purification protocol of Feuer *et al.* (1948) has remained essentially intact. With the advent of polyacrylamide gel electrophoresis as a highly resolving analytical tool for ascertaining protein purity, it became evident that muscle actin isolated by this classical procedure contained significant amounts of actomyosin-associated muscle proteins such as tropomyosin (Laki *et al.*, 1962) and $\alpha$-actinin (Ebashi and Ebashi, 1965). These contaminants promote the gelation of F-actin and greatly affect the physical properties of filaments in solution (Maruyama *et al.*, 1974). Drabikowski and Gergely (1962) showed that an enhanced purification could be achieved by extraction of the actin from muscle acetone powder at 0°C, and Spudich and Watt (1971) devised a modification of this method designed to eliminate tropomyosin from muscle actin preparations. Their purification resulted in a single band on SDS-polyacrylamide gels and has met with widespread use as a general method for obtaining muscle actin.

A subtle problem in establishing methods for actin purification resides in the level of purity acceptable for the investigations at hand. Emerging experimentation in cell biology and, specifically, cytoskeletal biochemistry requires probing sensitive properties of actin itself and actin associations with other cell proteins. It is therefore the goal of this review to explore some of the pitfalls associated with actin purification and to clarify in some detail the correct usage and expected result from each step of the widely used muscle actin purification proce-

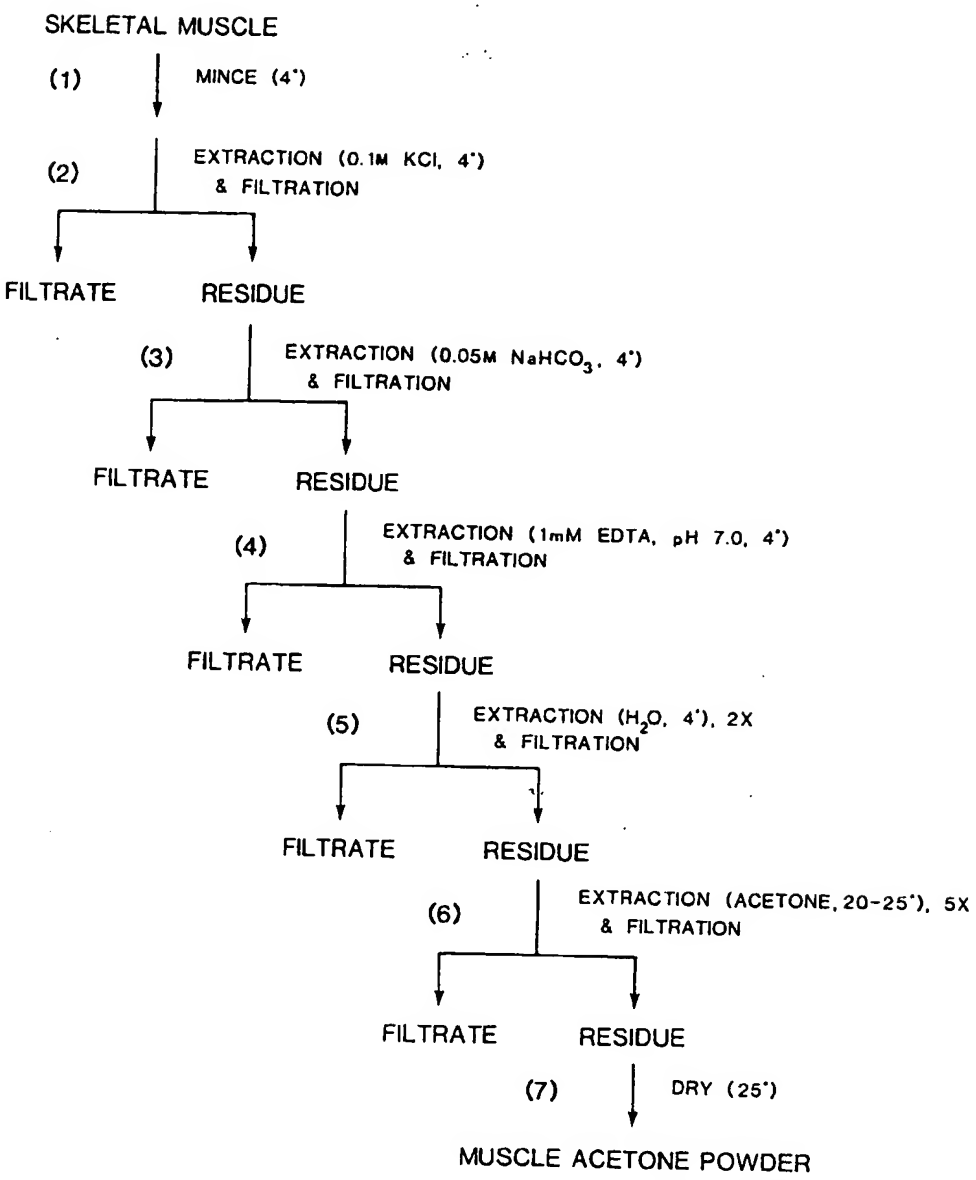


FIG. 2. Flow diagram for muscle acetone powder preparation. For complete explanation of the protocol, refer to steps in Section II of the text.

dure of Spudich and Watt (1971). Furthermore, additional steps to eliminate trace contaminants are described.

Flow diagrams for isolating rabbit skeletal muscle actin are given in Figs. 2 and 3. The detailed procedure for the acetone powder preparation of Feuer *et al.* (1948) with minor modifications is presented (Fig. 2) because of its importance in eliminating myosin and proteases from the final product and because the original reference may not be readily available. Adherence to requirements of temperature, buffer conditions, and incubation times are of prime importance for

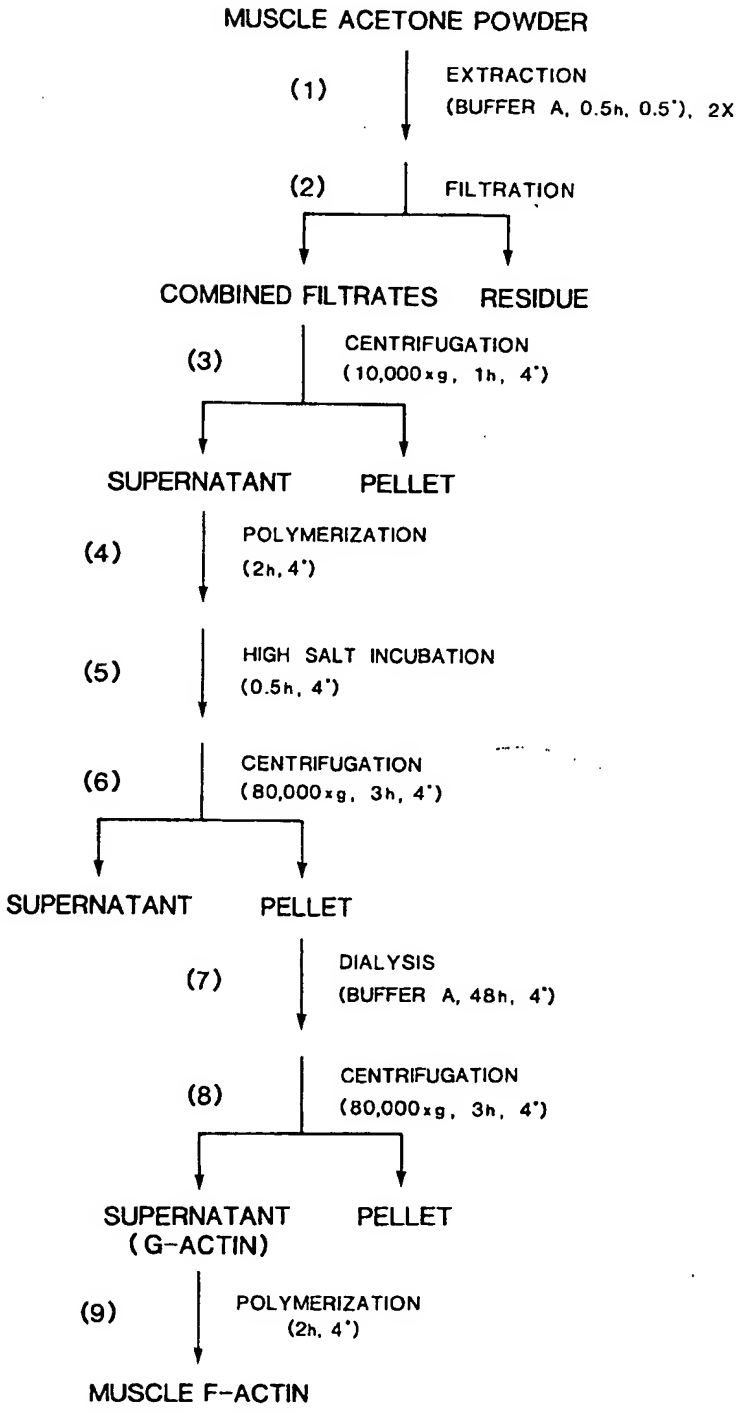


FIG. 3. Flow diagram for muscle actin isolation. For complete explanation of the protocol, refer to steps in Section III of the text.

obtaining high-purity actin. Steps at 4°C are preferably carried out in a cold room; cold extraction buffers and solvents are prechilled to 4°C before use, and buffer pH is determined at 25°C before chilling.

## II. Acetone Powder Preparation (Fig. 2)

*a. Preparation of Muscle Mince.* The preferred way to kill the rabbit is to let it hang head down by grasping the hind legs with one hand while delivering a sharp blow to the back of the neck, followed by bleeding the animal completely. Immediately after sacrifice, the dorsal lateral skeletal muscles and the hind leg muscles are excised (about 350 g), chilled on ice, washed clear of blood with distilled $H_2O$, and minced at 4°C in a prechilled meat grinder.

*b. Extraction with KCl.* The mince is quickly extracted with stirring for 10 min in 1 liter of ice-cold 0.1 *M* KCl, 0.15 *M* potassium phosphate, pH 6.5. All extracts are filtered by squeezing through four layers of cheesecloth which have been previously boiled for approximately 20 min in distilled $H_2O$, drained, and brought to 4°C.

*c. Extraction with $NaHCO_3$.* The filtered muscle mince is extracted with stirring for 10 min at 4°C in 2 liters of prechilled 0.05 *M* $NaHCO_3$ and filtered. Longer extraction times at this stage cause appreciable extraction of actin and are to be avoided (J. R. Bamburg, personal communication).

*d. Extraction with EDTA.* The filtered residue is extracted with 1 liter of 1 m*M* EDTA, pH 7.0, by stirring for 10 min at 4°C.

*e. Extraction with $H_2O$.* The next two extractions are with 2 liters of 4°C distilled $H_2O$ for 5 min with stirring.

*f. Extraction with Acetone.* The final five extractions are with 1 liter of acetone for 10 min each. All acetone extractions are performed at 20–25°C. Acetone should be cooled to below 20°C or the acetone–mince mixture becomes too warm. Clumps of residue are broken up by stirring during each extraction.

*g. Drying.* The filtered residue is placed in large glass evaporating dishes and air-dried overnight in a hood to obtain dried "acetone powder." The resulting acetone powder is stable for months if stored at −20°C.

## III. Actin Isolation (Fig. 3)

Typical preparations use about 10 g of acetone powder. The minimal yield is approximately 10 mg actin per gram acetone powder, but can be as high as 30 mg actin per gram acetone powder.

## A. Extraction

The acetone powder is extracted at 0–0.5°C for 30 min by stirring with 20 ml buffer A per gram acetone powder. The temperature must be kept low during the extraction (Drabikowski and Gergely, 1962). Buffer A consists of 2 m*M* Tris-Cl, 0.2 m*M* $Na_2ATP$, 0.5 m*M* 2-mercaptoethanol, 0.2 m*M* $CaCl_2$, 0.005% azide, final pH = 8.0 at 25°C.

Because actin is extracted under depolymerizing conditions, it is desirable to keep the concentrations of $Mg^{2+}$, $K^+$, and $Na^+$ in the buffer as low as possible. Measurable actin assembly occurs in 2 m*M* $K^+$ or $Na^+$, and in 0.2 m*M* $Mg^{2+}$ (Pardee and Spudich, 1982). Therefore, it is useful to employ reagent powders of Tris-base, $K_2ATP$ or $Na_2ATP$, and $CaCl_2$. To prepare the buffer, the reagents are dissolved in double distilled $H_2O$ and titrated to pH 8.0 at 25°C with HCl. Add 2-mercaptoethanol after the pH determination, since 2-mercaptoethanol (and dithiothreitol) interferes with accurate pH determination by impairing the sensitivity of the pH electrode. The result is drifting pH readings.

An important caution is that actin is susceptible to proteolysis resulting from even minor bacterial contamination. Therefore, if buffers are prepared from stock solutions rather than from reagent powders, buffer stocks should be stored at 4°C with 0.1% sodium azide present to prevent bacterial or mold growth.

## B. Filtration

The extract is separated from the hydrated acetone powder by squeezing through several layers of sterile cheesecloth; latex gloves are used to avoid contamination. Filtration through a coarse sintered glass filter under vacuum can also be used, but if sufficient care is not taken, foaming of the protein filtrate will occur, resulting in actin denaturation and reduced yields. If necessary, low-speed centrifugation at 5000–10,000 *g* for 10–20 min readily removes the bulk of the solids. Reextract the residue by stirring 10 min in 20 ml buffer A per gram acetone powder. Filter and combine extracts.

## C. Centrifugation

The extract is centrifuged at 20,000 *g* for 1 hr at 4°C. Decant the supernatant by hand pipetting, leaving the turbid lower layer in the centrifuge tube.

## D. Polymerization

The KCl concentration of the supernatant is brought to 50 m*M*, $Mg^{2+}$ to 2 m*M*, and ATP to 1 m*M*. Inclusion of 1 m*M* ATP at this step ensures full polymerization. Allow to assemble for 2 hr at 4°C. At this stage of isolation a visible increase in the solution viscosity should be observed.

## E. High Salt Wash: Tropomyosin Removal

Solid KCl is slowly added with stirring to a final concentration of 0.6 *M*, and the solution is stirred gently for 0.5 hr. Some investigators (MacLean-Fletcher and Pollard, 1980) have successfully employed 0.8 *M* KCl at 4°C in the wash step, which is useful in the event that the 0.6 *M* KCl treatment does not eliminate tropomyosin from the actin preparation. However, the actin monomer concentration increases with increasing salt concentration above 0.15 *M* (Kasai, 1969); thus lower yields of actin may result from washes with higher concentrations of salt.

## F. Sedimentation of Filamentous Actin

The polymerized actin is centrifuged in 30-ml tubes at 80,000 *g* (ave) for 3 hr at 4°C. To obtain optimal purity it is advisable to remove contaminants trapped in the liquid phase of the F-actin pellet. This can be achieved by homogenizing the total pelleted F-actin into 150 ml of fresh wash buffer (buffer A + 0.6 *M* KCl, 2 m*M* $MgCl_2$, 1 m*M* ATP) and resedimenting the F-actin at 80,000 *g* (ave) for 3 hr at 4°C.[1] After discarding the supernatant, the intact F-actin pellet is rinsed thoroughly with buffer A.

## G. Depolymerization

The pellets of F-actin are resuspended by gentle homogenization in 3 ml of cold buffer A per gram acetone powder originally extracted. Large actin losses can occur because of incomplete transfer to the homogenizer. A good technique to maximize recovery of F-actin from the centrifuge tube is to allow each pellet to stand on ice in 1 ml of buffer A for 1 hr before transferring to the homogenizer. The softened pellets can then be partially homogenized with a Teflon-coated rod and transferred with a plastic disposable pipette to the homogenizer without significant losses. Dialysis at 4°C against 1 liter of prechilled buffer A with one or two changes over a 3-day period gives complete depolymerization of actin, although dialysis times can be shortened considerably if vigorous stirring and large surface area dialysis bags are employed. One technique is to divide the homogenate into equal 6-ml aliquots (for 10 g acetone powder extracted) and place them into dialysis bags of ¼-in. diameter × 12 in. long. The bags are then either mounted on a rapid dialyser or tied to a magnetic stir bar in a 1-liter graduated cylinder. Rapid rotation of the dialysis bag permits nearly complete exchange of solutes in approximately 6 hr. Three buffer changes at 12-hr intervals results in >90% depolymerization of the F-actin (Fig. 4).

[1]This additional wash was not included as a step in the original Spudich-Watt report (Spudich and Watt, 1971) and was not used to purify the actin shown in the figures presented here. This has now been incorporated as a routine step in the procedure.

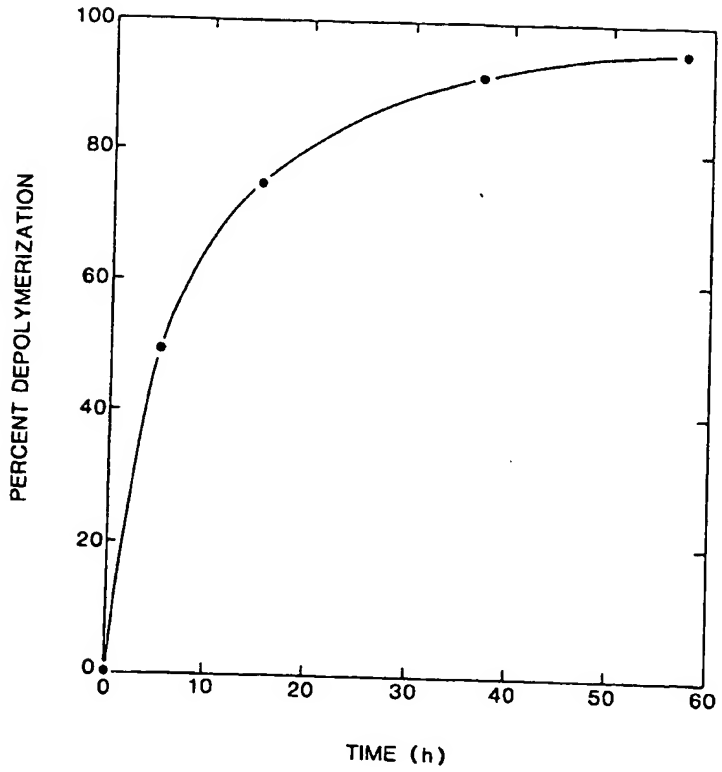


FIG. 4. Time course of depolymerization. Aliquots of dialysing actin from step 7 of the isolation procedure (Fig. 3) were centrifuged at 100,000 *g* (ave) for 2 hr at 4°C. Protein was determined in the supernatant (depolymerized actin), and % depolymerization was calculated. Dialysis was performed in 12-in. × ¼-in. dialysis bags mounted on a rapid dialyser in 1 liter of buffer A at 4°C. The buffer was changed at 5, 15, and 37 hr of dialysis. Total dialysis time was 57 hr.

It is not unusual to detect a residual viscosity in the dialysed actin. This viscosity is due to the presence of nondepolymerized actin which is complexed with myosin or other proteins. The quantity of myosin present can vary from preparation to preparation as a result of differing efficiencies of myosin removal during acetone powder preparation. Some actin losses are encountered with short dialysis times, but a myosin-free final product is obtained. Extensive dialysis results in eventual dissociation of actin–myosin complexes and appearance of myosin in the final product. Prolonged dialysis can also lead to observable actin proteolysis, and should therefore be avoided.

## H. Clarification of G-Actin

The dialysed actin is centrifuged at 80,000 *g* (ave) for 3 hr. Shorter centrifugation times can be employed at greater *g* force; e.g., 150,000 *g* (ave) is now readily attainable in modern ultracentrifuges with the corresponding clearing

time reduced to 1.5 hr. The supernatant fraction is saved. It is convenient to determine protein concentration at this point rather than on the subsequent viscous F-actin final product.

## I. Polymerization

G-actin solutions, even in the presence of high concentrations of ATP (1 m*M*) and stored on ice, begin to lose polymerization activity after 2–3 days. Therefore, actin is stored as F-actin. To polymerize, add KCl to 50 m*M*, $MgCl_2$ to 1 m*M*, and ATP to 1 m*M* final concentrations. For storage, also add 0.02% $NaN_3$. The final product can be stored on ice as an F-actin solution or as pellets of F-actin. Pelleting provides additional stability. Actin should not be frozen or lyophilized.

The expected yield for this protocol is 20–30 mg actin per gram acetone powder. The resulting actin is generally highly purified (Fig. 5). However, depending upon individual technique, buffer purity, dialysis times employed, and so forth, the actin preparation can contain small amounts of contaminating protein, including proteolysed actin. Consequently, several techniques for further purification of the actin are discussed in Section VI.

## IV. Analysis of Purity of Final Product

Contaminating proteins are most easily detected by SDS–polyacrylamide gel electrophoresis (Laemmli, 1970; Ames, 1974), preferably utilizing slab-type gels of 1.5–2.0 mm thickness. Our experience is that 10–12% acrylamide is an optimal gel concentration for detection of contaminants, since in 8% gels protein species of <25,000 daltons run with the ion front, whereas 15% gels do not allow discrimination of high-molecular-weight proteins. The limit of detection for Coomassie Blue stained protein bands on a 1.5-mm-thick slab gel system is about 0.05 $\mu$g/band. Consequently, visualization or densitometry of one 0.15% contaminant requires loading approximately 40 $\mu$g of the actin preparation (Fig. 6). Although this constitutes gross overloading of the actin band (actin band staining is linear only to 4 $\mu$g/band), minor contaminations of 0.15% can be detected and purity estimated. It is highly desirable to have this degree of sensitivity since very low levels of other components in actin preparations can significantly alter the properties of actin filaments (MacLean-Fletcher and Pollard, 1980). For example, a factor that alters the function of actin filaments by specifically binding to filament ends need only represent about 0.2% of the protein in a preparation of filaments about 1 $\mu$m long.

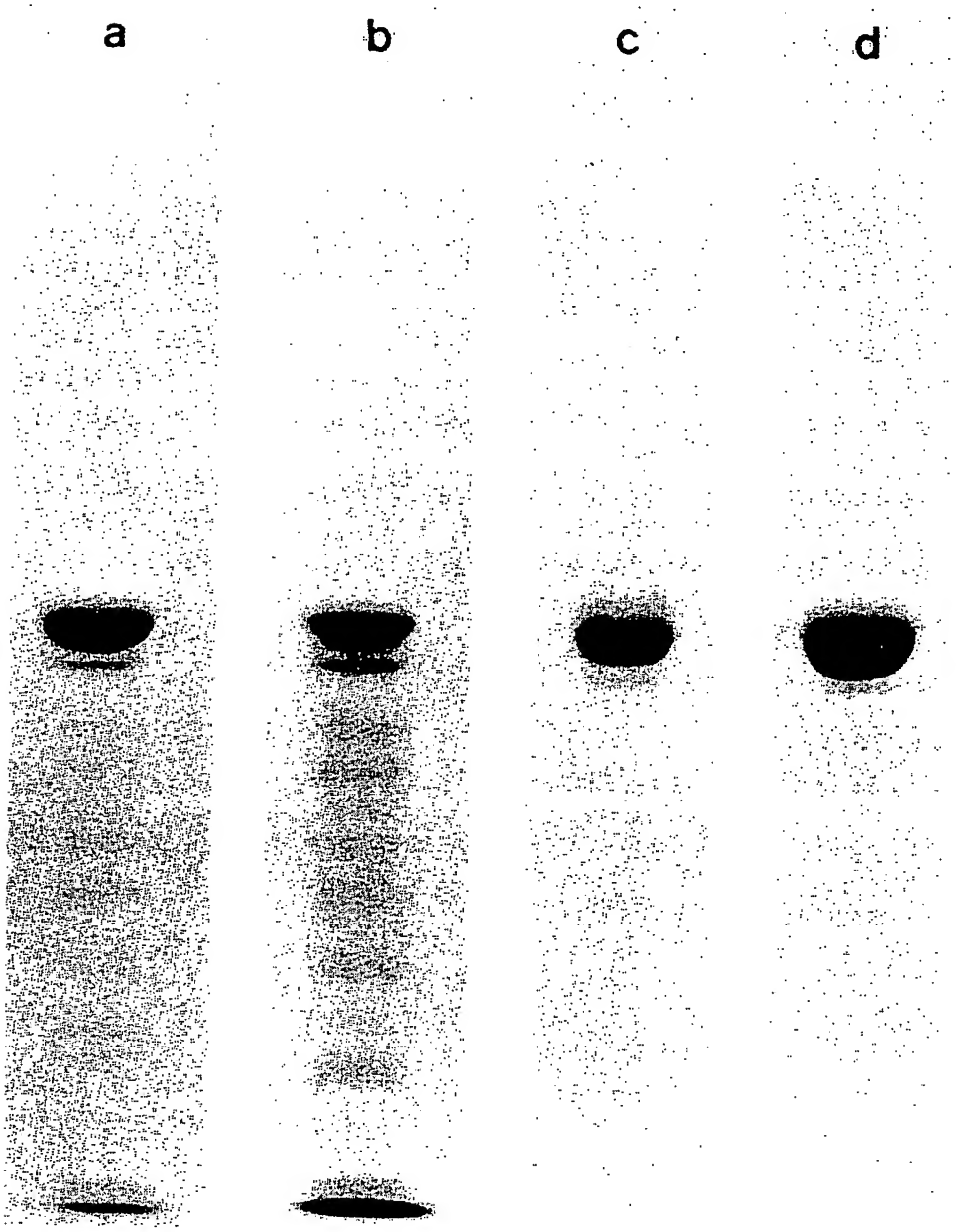


FIG. 5. SDS-PAGE of fractions at various stages in the actin purification. Samples taken during the isolation procedure were mixed 1:1 with a solution containing 2% SDS, 1% 2-mercaptoethanol, boiled for 3 min and applied to a 1.5-mm-thick slab gel containing 12% acrylamide, 0.2% methylene bisacrylamide, and 0.1% SDS in the buffer system of Laemmli (1970). Gels were stained overnight with 0.025% Coomassie Brilliant Blue G, 10% acetic acid, 25% isopropanol, and destained in 10% acetic acid by gentle shaking. Each gel lane contains about 6 $\mu$g of protein. (a) Muscle extract after 10,000 *g* clarification (Fig. 3, step 3). (b) Supernatant after incubation in 0.6 *M* KCl and centrifugation at 80,000 *g* (step 6). (c) Sedimented F-actin after incubation in 0.6 *M* KCl (step 6). (d) Final product (step 9).

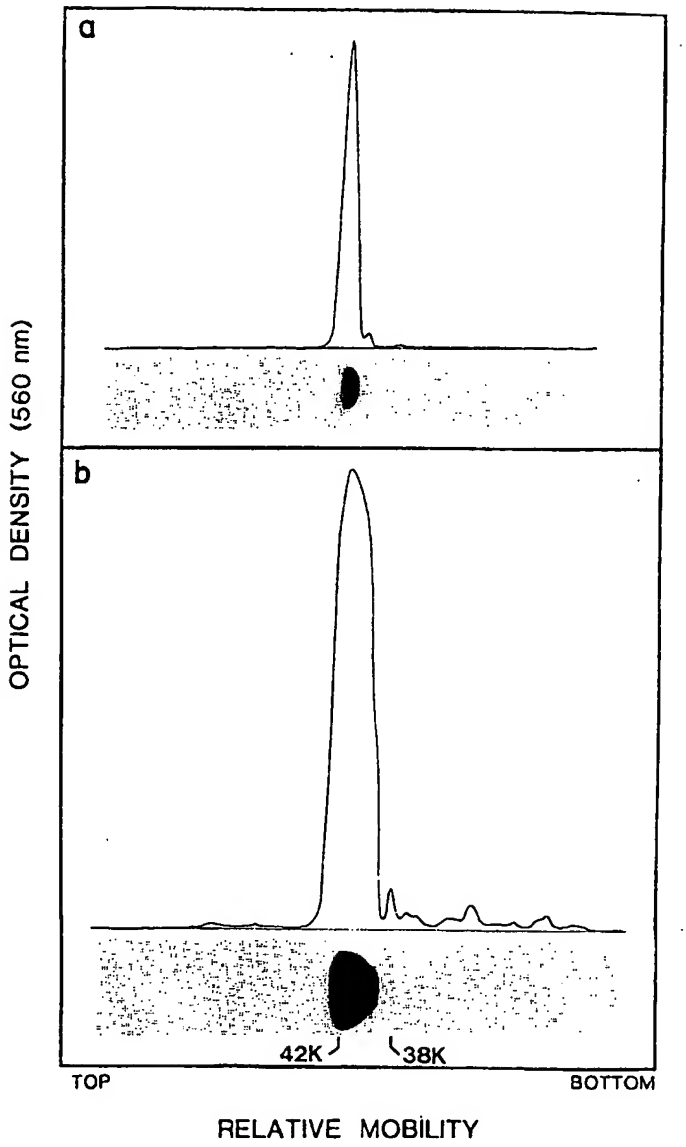


FIG. 6. Detection of actin contamination. Actin prepared as in Fig. 3 was electrophoresed in a 12% SDS-polyacrylamide gel. Stained gels were scanned at 560 nm on a RFT scanning densitometer (Transidyne). (a) 4 $\mu$g of isolated actin. Homogeneity was estimated at 98% by scan area. (b) 40 $\mu$g of isolated actin. Approximately eleven contaminants containing a total of about 2 $\mu$g of protein are detected by densitometry. Homogeneity $\cong$ 95%.

## V. Sources of Contamination

Myosin contamination is sometimes observed and can be attributed to incomplete myosin extraction during acetone powder preparation. Proteolysis of actin

is indicated by the appearance of a 38,000 dalton protease-resistant core (Jacobson and Rosenbusch, 1976) (Fig. 6b). Such proteolysis can arise either from bacterial contamination in extraction buffers or from proteolytic activity extracted with actin from some acetone powders (Fig. 7). While proteases are not prevalent in all acetone powder preparations, inspection of purified actin prepa-

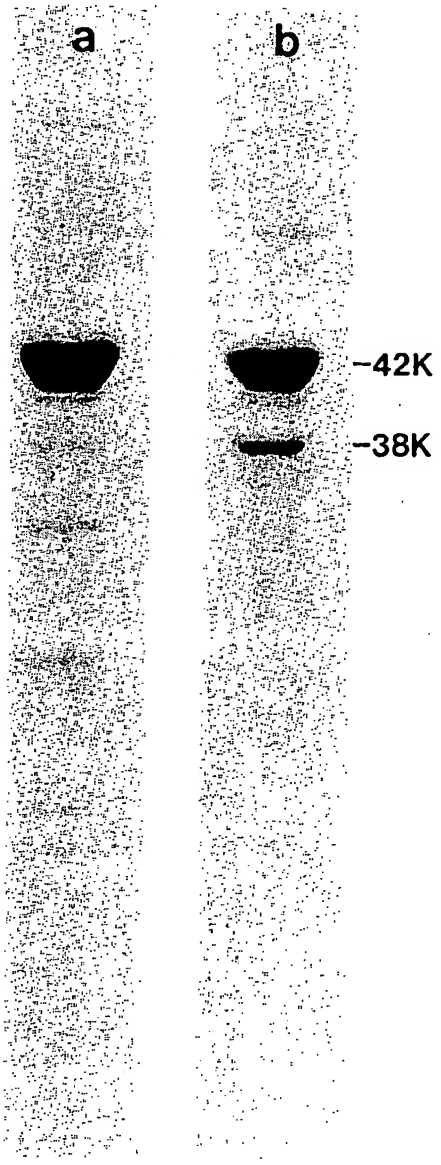


FIG. 7. Proteolytic activity in acetone powder extracts. (a) Acetone powder extract (10,000 *g* supernatant; Fig. 3, step 3) taken immediately after extraction. Note the presence of a small amount of 38,000-dalton proteolytic fragment. (b) The same acetone powder extract after 24-hr incubation at 20°C. Significant proteolysis of actin has occurred, resulting in a larger amount of the 38,000-dalton fragment. Not all of the acetone powders tested contained an active protease.

rations for protease activity is advisable. For those acetone powders that yield proteolytic activity, a slight modification of the actin preparation procedure is recommended. After sedimenting F-actin following the 0.6 *M* KCl treatment (see Fig. 3, step 6), the F-actin pellet is homogenized thoroughly into 100 ml of 4°C buffer A containing 50 m*M* KCl and 2 m*M* $MgCl_2$ and immediately recentrifuged at 150,000 *g* for 1.5 hr at 4°C. The soluble protease is fractionated away from F-actin in this step before depolymerization of filaments into protease-susceptible G-actin has been initiated. The washed pellet is then homogenized into buffer A and dialysed (step 7). An additional precaution when the acetone powder preparation contains proteolytic activity is to ensure that the pH of the extraction buffer is 8.0 at 25°C; high pH inhibits protease activity.

## VI. Further Purification of Actin

Many current experiments in research on cell motility require actin completely free of trace contaminants such as myosin, tropomyosin, and other factors that are known to alter properties of actin assembly, disassembly, exchange, and ATPase activity. In addition, no specific steps in the purification shown in Fig. 3 are designed to efficiently remove ribonucleotides or polysaccharides, which are not detected by SDS-gel electrophoresis with Coomassie Blue staining. Consequently, we and others have designed additional steps to further purify the actin. The three following procedures can be considered alternatives or they can all be used.

### A. Ion Exchange Chromatography

A highly recommended technique for obtaining highly purified muscle actin is ion exchange chromatography. This type of purification offers the considerable advantage of removing both protein and nonprotein contaminants from actin preparations. For further purification of muscle actin we use the following batch treatment of F-actin with DEAE cellulose.

1. DE-52 resin (Whatman) is prepared at 25°C in 50 m*M* triethanolamine buffer, pH 7.5, following the instructions provided with the resin. Two ml of settled resin are used to further purify 20 mg of actin isolated as shown in Fig. 3.
2. Two ml of settled resin are sedimented at 20,000 *g* for 20 min at 4°C, and resuspended with 200 ml of DEAE-buffer (10 m*M* imidazole, pH 8.0, at 4°, 0.1 *M* KCl, 0.1 m*M* $CaCl_2$, 1 m*M* ATP, 0.5 m*M* 2-mercaptoethanol, and 0.005% $NaN_3$). Equilibration is for 4 hr at 4°C with stirring.
3. Equilibrated resin is sedimented and resuspended with 20 ml of 1.0 mg/ml F-actin, which is prepared by diluting F-actin (~5 mg/ml) with cold DEAE-buffer and mixing for 30 min at 4°C.


FIG. 8. DEAE purification of isolated actin. F-actin (20 mg) isolated as in Fig. 3 was treated with DE-52 by the batch method described in Section VI,A. (a) Isolated actin before DEAE treatment. Densitometry of contaminants indicates 1–2 $\mu$g of protein contamination per 40 $\mu$g of loaded protein, or about 95% actin homogeneity. (b) Isolated actin after DEAE purification. Densitometry of contaminants indicates <0.2 $\mu$g of contamination per 40 $\mu$g of loaded protein. Molecular weight homogeneity >99%.

4. The F-actin, DE-52 mixture is stirred in a plastic beaker for 6 hr at 4°C. More than 95% of the actin is loaded onto the DE-52 in this step.

5. The resin is sedimented at 20,000 *g* for 20 min at 4°C and the supernatant is discarded.

6. The resin is resuspended by stirring in 20 ml of 10 m*M* imidazole, pH 6.4, at 4°C, 0.3 *M* KCl, 0.1 m*M* $CaCl_2$, 1 m*M* ATP, 0.5 m*M* 2-mercaptoethanol, and 0.005% azide. Actin is eluted for 2 hr at 4°C by mixing.

7. The resin is sedimented (20,000 *g*, 20 min, 4°C) and the supernatant (20 ml) is immediately dialysed against 2 liters of buffer A containing 50 m*M* KCl, 2 m*M* $MgCl_2$, and 1 m*M* ATP.

8. The dialysed F-actin is pelleted at 150,000 *g* for 1.5 hr at 4°C, homogenized into buffer A to a final actin concentration of 6–7 mg/ml, and depolymerized by overnight dialysis.

9. The resulting G-actin is clarified at 150,000 *g* for 1.5 hr at 4°C, assembled with 0.1 *M* KCl and 1 m*M* $MgCl_2$, and stored on ice in the presence of 0.005% sodium azide.

The recovery from this procedure is approximately 50% with a final product purity of greater than 99% (Fig. 8).

## B. Depolymerization–Repolymerization

The actin can also be further purified by the following recycling protocol (Fig. 9). We often carry out this procedure just prior to using the actin in an experiment.

1. F-actin (stored as a viscous solution at 4–6 mg/ml) is diluted to 0.5 mg/ml and allowed to incubate at 4°C for 2 hr in the presence of 0.1 *M* KCl, 1 m*M* $MgCl_2$, and freshly added 1 m*M* ATP. Fresh ATP is always added to stored actin just prior to recycling. Incubated F-actin is sedimented at 150,000 *g* for 1.5 hr at 4°C, the supernatant decanted, and the tube and pellet rinsed carefully with buffer A.

2. The resulting F-actin pellet is rinsed carefully with buffer and gently homogenized into cold buffer A (see Section III,G) to a final actin concentration of 2–4 mg/ml and dialysed against 1 liter of buffer through a 10,000 dalton cutoff collodion bag (Schleicher and Schull) for 6 hr at 4°C with rapid stirring. Collodion bags are much more permeable to ATP in low-ionic-strength buffers than the traditionally used cellulose dialysis tubing (see Pardee and Spudich, 1982; Martonosi *et al.*, 1960). Consequently, actin depolymerization rates are enhanced and G-actin denaturation resulting from depletion of ATP within the dialysis bag is minimized. It is important to minimize dialysis time since pure actin depolymerizes quickly while actin associated with contaminants such as myosin and gelation factors depolymerizes more slowly; the basis of this purification step resides in these differential depolymerization rates.

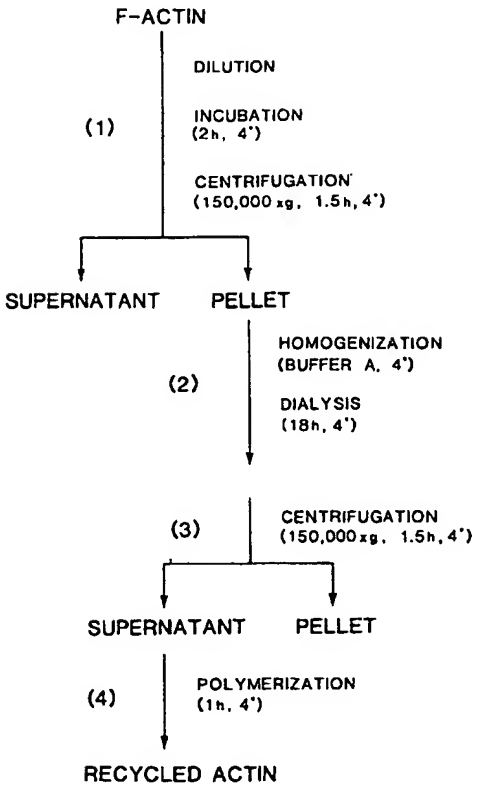


FIG. 9. Flow diagram for recycling the actin isolated as in Fig. 3. For complete explanation of the procedure, refer to the numbered steps in Section VI,B of the text. Dialysis is performed in 10,000 dalton cut-off collodion bags. Recovery is approximately 70% of starting material.

3. Dialysed actin (which still may retain a visible viscosity if actin associated contaminants are present in the starting material) is centrifuged at 150,000 *g* for 1.5 hr at 4°C, the supernatant is decanted, and any pelleted material is discarded.

4. The clarified G-actin is immediately polymerized by addition of ATP, KCl, and $MgCl_2$ to final concentrations of 1 m*M*, 0.1 *M*, and 1 m*M*, respectively. Full assembly is complete within 1 hr at 4°C. The F-actin solution at this stage should be quite clear and highly viscous. (The actin concentration is approximately 2 mg/ml). Purification may be complete at this stage, depending on the purity and type of contaminants originally present. However, a second recycling may be necessary when small amounts of proteolysed actin and low-molecular-weight contaminants persist.

The yield from this procedure depends on the level of contamination present in the starting actin material. For highly contaminated starting material (purity = 85–90%), approximately 50% recovery of highly purified actin is obtained. For actin that is 95% pure, recovery is approximately 75%. The value of recycling even apparently clean actin preparations is illustrated in Fig. 10. Although the

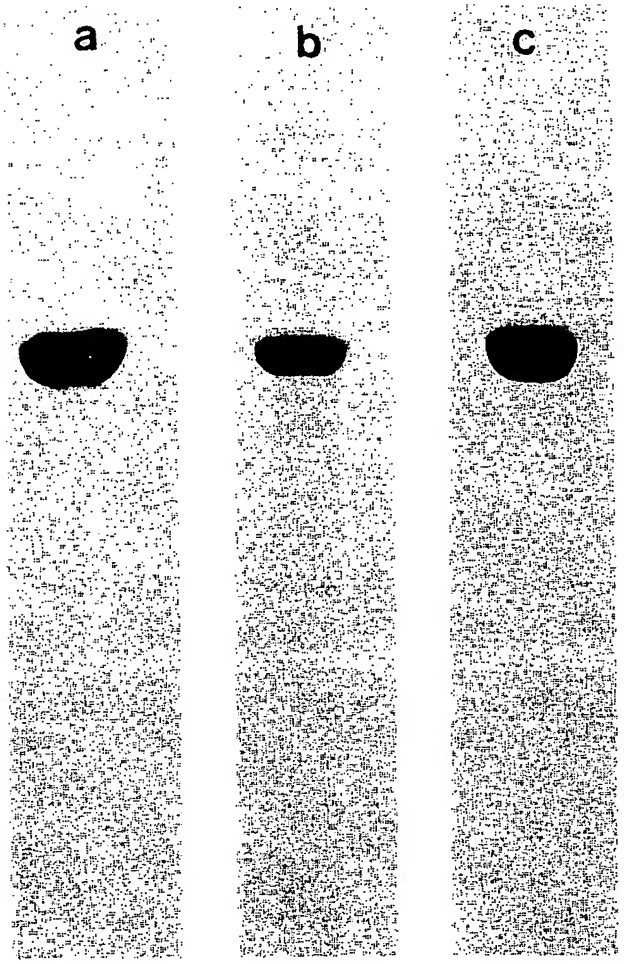


FIG. 10. Removal of F-actin-associated contaminants by recycling. (a) Actin from the isolation procedure shown in Fig. 3. (b) Sedimented material after depolymerization of the F-actin (Fig. 9, step 3). (c) Final product after recycling. Each lane was loaded with 20 $\mu$g of protein.

purity of the actin before recycling is high, the F-actin-associated impurities removed by a single recycling procedure are evident in the depolymerization pellet (Fig. 10b).

## C. Sephadex Chromatography

G-150 Sephadex chromatography in depolymerization buffer can be used to provide additional purification as well as removal of actin oligomers from G-actin. Polymers are easily separated from the major peak of low-molecular-weight actin species, and if the trailing fractions of the eluted actin peak are pooled, a homogeneous population of monomeric actin can be obtained in addi-

tion to increasing the actin purity. The effects of purification by G-150 Sephadex on the properties of muscle actin have been studied recently by MacLean-Fletcher and Pollard (1980). Polymerization of column purified monomeric actin results in a significant increase in low-shear viscosity over that observed with actin before column purification. Moreover, some of the fractions from the Sephadex column show a significant viscosity-reducing activity when added back to the column-purified G-actin before polymerization. These results should be sufficient to warn of dramatic effects on the properties of actin by levels of contaminants heretofore considered negligible.

## VII. Concluding Remarks

This review is devoted to the purification of muscle actin for two reasons. First, muscle actin has become a common laboratory reagent, and we therefore wished to discuss the details of the purification procedure of Spudich and Watt (1971) with special reference to possible problems that may be encountered. Second, the current level of sophistication in cell motility research places new demands on the level of acceptable purity of the actin preparation. Thus several additional steps are presented for further purification of muscle actin. Particularly useful is a simple batch treatment of F-actin with DEAE-cellulose, which gives good recovery of actin that is >99% pure.

In the last decade, actin from many cell types has become widely studied (for reviews, see Pollard and Weihing, 1974; Goldman *et al.*, 1976; Pollard, 1977; Weihing, 1976; Clarke and Spudich, 1977; Korn, 1978). Optimal purification of nonmuscle actins generally requires procedures specifically tailored to the cell type used (Yang and Perdue, 1972; Spudich, 1974; Hartwig and Stossel, 1975; Kane, 1975; Pollard *et al.*, 1976; Gordon *et al.*, 1976; Sheetz *et al.*, 1976; Hatano and Owaribe, 1977; Adelman, 1977; Uyemura *et al.*, 1978; Pardee and Bamburg, 1979; Weir and Frederiksen, 1980). An evaluation of various procedures for the purification of nonmuscle actins can be found in a recent review by Uyemura and Spudich (1980).

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